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4862 '99 DEC 20 A9:39

December 16, 1999

Mr. Lyle D. Jaffe
Dockets Management Branch
Food and Drug Administration
Department of Health and Human Services
5630 Fisher's Lane
Room 1061
Rockville, MD 20852

Dear Mr. Jaffe:

Per our conversation this morning, I am submitting the attached addenda in support of the Citizens Petition for the Proposed Amendment to Classification and Product Labeling for the Sympathominetic Amine Phentermine, FDA docket number 99P-4053/CP 1, originally filed on September 10, 1999.

The attachments consist of five (5) copies each of (a) Biochemical Pharmacology's letter to Dr. Richard J. Wurtman, informing him that his paper, "Characterization of Phentermine and Related Compounds as Monoamine Oxidase Inhibitors (MAOI)," authored by Ulus IH, Maher TJ and Wurtman RJ, has been accepted for publication; and (b) the article itself, copies of which were in our original citizens petition.

The article will appear in the next few months of Biochemical Pharmacology and is further evidence of scientific validation for this petition and for an affirmative decision by the FDA in this matter.

Please feel free to contact me if you need additional information or have any questions.

Sincerely,

Mark P. McGrath

99P-4053

SUP 2

Biochemical Pharmacology

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November 23, 1999

Dr. Richard J. Wurtman
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Technology
77 Massachusetts Ave., E25-604
Cambridge, MA 02139

Dear Dr. Wurtman:

Re: 252-3211-9-Revised-2, "Characterization of phentermine...," by I.H. Ulus et al.

Thank you for sending your revised manuscript to us. It is my pleasure to inform you that your paper is now acceptable for publication in *Biochemical Pharmacology.*

Proofs will be sent to you from Elsevier Science in New York in approximately three months. Please compare your edited manuscript and the galley proofs, checking carefully to see that they conform in every way and that there are no errors.

Enclosed please find a transfer of copyright agreement form for you to complete and return to us as soon as possible.

We look forward to the early publication of your manuscript.

Sincerely,

Robert H. Roth
Associate Editor

RHR:sed
Enclosure

ELSEVIER

Submitted to
Biochem. Pharm.
(1999)

CHARACTERIZATION OF PHENTERMINE AND RELATED COMPOUNDS AS MONOAMINE OXIDASE INHIBITORS (MAOI)

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RUNNING TITLE: Phentermine Inhibits Monoamine Oxidase

PAPER CLASSIFICATION: Cardiovascular and Pulmonary Pharmacology

ABSTRACT

Phentermine was shown in the 1970's to inhibit the metabolism of serotonin by monoamine oxidase (MAO), but never was labeled as an MAO inhibitor; hence it was widely used in combination with fenfluramine, and continues to be used, in violation of their labels, with other serotonin-uptake blockers. We examined the effects of phentermine and several other unlabeled MAO inhibitors on MAO activities in rat lung, brain, and liver, and also the interactions of such drugs when administered together.

Rat tissues were assayed for MAO A and B, using serotonin and phenylethylamine as substrates.

Phentermine inhibited serotonin-metabolizing (MAO A) activity in all three tissues with K_i's of 76-89 micromolar. These K_i's were similar to those of the antidepressant MAO inhibitors iproniazid and moclobemide. When phentermine was mixed with other unlabeled reversible MAO inhibitors (e.g., pseudoephedrine, ephedrine, norephedrine; estradiol benzoate) the degree of MAO inhibition was additive.

Serotonin is cleared from the plasma by uptake into tissues (principally lung and platelets) and by MAO A; fenfluramines block serotonin's uptake, and phentermine, as shown here, blocks its enzymatic degradation. The cardiac valvular lesions and primary pulmonary hypertension that have, rarely, been associated

with fen-phen use may have resulted from the intermittent concurrent blockage of both of these pathways.

KEY WORDS: Phentermine, MAO-A, MAO-B, Serotonin, Amines Anorectics, Cardiac Valvular Lesion

The anorexigen fenfluramine and its dextro-isomer dexfenfluramine have been implicated in the development of echocardiographically-defined cardiac valvular disease [1,2]. Initial open-label observations were interpreted as showing that as many as 30% of patients who took one of these drugs would develop such disease [2], however subsequent controlled studies indicate that it occurs much less frequently, and in most cases is asymptomatic [3-6].

Dexfenfluramine, which enhances serotonin- mediated neurotransmission by blocking serotonin's reuptake and, through its nordexfenfluramine metabolite, directly releasing the transmitter and activating postsynaptic 5HT-2A and -2C receptors [7], had been used for many years outside the United States, without association with valvular lesions; similarly, fenfluramine had been prescribed for three decades without generating known valvular pathology. Hence we wondered whether the occurrence of apparent valve damage in the United States, but not elsewhere, might have been related to the widespread, peculiarly American practice of taking fenfluramine along with another anorexigen, phentermine [8]. Phentermine, like fenfluramine but not dexfenfluramine, is a generic drug; it is usually described as a "sympathomimetic amine", implying a primary action on noradrenergic neurotransmission[9]. We examined

its effects in rats, and found that it releases brain dopamine [10], so we conducted additional studies to see whether it also raised blood dopamine levels in people [11].

After a single oral phentermine dose plasma dopamine levels did rise significantly, however levels of serotonin within blood platelets increased by an even greater proportion [11]. Since there was no concurrent rise in plasma serotonin levels, the increase in platelet serotonin most likely reflected slowed degradation of the amine, and not an increase in its uptake from the plasma. (Platelets are unable to synthesize serotonin.) This degradation is catalyzed in platelets by an enzyme that shares properties with the type of monoamine oxidase (MAO-B) that, in other organs, preferentially metabolizes phenylethylamine and related amines but not serotonin [12,13]. A literature search then revealed that phentermine's ability to inhibit MAO had been described in the 1970's [14,15], but, surprisingly, this property was not and still is not mentioned on the drug's label[9]. Thus even though the labels for fenfluramines and other serotonin-reuptake blockers instructed users never to take these compounds in combination with an MAO inhibitor[9], it had been possible for millions of Americans to take the "fen/phen" combination.

In early studies that described the inhibition of MAO by phentermine, the characteristics of this inhibition (e.g., its selectivity for MAO-A and MAO-B; its reversibility) were not

investigated fully. To understand the mechanism of phentermine's pharmacological effects and its interactions with other drugs, we have now characterized its inhibition of MAO in rat brain and liver, and in lung, the site at which most plasma serotonin is destroyed [16]. The drug inhibits the enzyme in all three tissues, with a potency similar to those of the antidepressant MAO inhibitors iproniazid and moclobemide. Moreover numerous other widely-used drugs also are unlabeled MAO inhibitors, and the effects of administering two or more of them together can be additive.

MATERIALS AND METHODS

Determination of MAO-A and MAO-B activities. Male Sprague-Dawley rats (Taconic) weighing 250-275 g were decapitated and their lungs, liver, and brain removed, rinsed with saline solution (0.9% NaCl), dried on filter paper, weighed, and homogenized in 10 (lung) or 20 (liver and brain) volumes of 0.1 M sodium phosphate buffer. MAO-A and MAO-B activities were assayed as described previously by Lyles and Callingham [17] with some minor modifications. (We assume that the serotonin-metabolizing enzyme in these tissues, unlike that in human platelets, is MAO-A; hence we use this term and serotonin-metabolizing activity, or MAO-B and phenylethylamine-metabolizing activity, interchangeably.) Briefly, 25 μl of homogenate were

preincubated in a round-bottom glass culture tube (10-13x75 mm) for 20 min at 37°C in the absence or presence of various concentrations (typically 6-8) of a compound to be tested, in a total volume of 50 *µl*. Following preincubation, the reaction was started by adding 50 *µl* of [^{14}C]-serotonin (final concentration 125 µM in experiments for determining IC_{50}'s or 32-2000 µM for kinetic studies) or [^{14}C]-phenylethylamine (final concentrations 8 µM in experiments for determining IC50's or 1-128 µM for kinetic studies). Incubation times were 5 or 10 min for studies on [^{14}C]-serotonin and 2 min for those on [^{14}C]-phenylethylamine. Reactions were stopped by the addition of 25 *µl* of 3 N HCl. Deaminated metabolites were extracted by vigorous vortexing for 20-30 seconds with 1 ml of toluene: ethylacetate (1:1;v:v) saturated with water. Following extraction the tubes were centrifuged for 10 min (1500xg, + 4°C) to separate the aqueous and organic phases. The aqueous phase was then frozen on dry ice and the organic phase collected into a small (7 ml) scintillation vial for subsequent counting. After addition of 5 ml of Opti-fluor® and vigorous shaking, radioactivity was measured with a liquid scintillation spectrometer (LS-6500, Beckmann, Irvine CA, USA). Blank values were obtained by adding 25 *µl* of 3 N HCl to the enzyme mixture prior to adding the substrate.

In studies on the inhibition of MAO activities by

phentermine and other drugs, 25 μl aliquots of rat brain, liver and lung homogenates were preincubated for 20 min at 37°C in the absence or presence of various concentrations of phentermine or other test compounds, in a total volume of 50 μl. Following preincubation, the reaction was started by addition of 50 μl of [^{14}C]-serotonin or [^{14}C]-phenylethylamine, and samples were subsequently treated as above. Enzyme activities observed with each concentration of a test compound were expressed as percents of the activity observed in the absence of the drug. For each compound the concentration-inhibition curve was obtained by plotting the log molar concentration of a test compound against the percent of control enzyme activity remaining. The concentration of a test compound producing 50% inhibition (IC_{50}) was calculated graphically from semi-log plots (the concentration-inhibition curve) of inhibitor concentration against percentage inhibition. K_i values (μM) were determined from the equation $K_i = IC_{50}/(1 + S/Km)$. The S value (substrate concentration as μM) in this equation was 125 μM for [^{14}C]-serotonin or 8 μM for [^{14}C]-phenylethylamine, respectively. K_m values (μM) for serotonin or phenylethylamine were obtained for each tissue from the double-reciprocal Lineweaver-Burk plots of data from kinetic experiments. In these experiments, 25 μl of homogenate were preincubated for 20 min in the absence of drug in a total volume of 50 µl (25 μl of homogenate + 25 μl of water).

Following preincubation, the reaction was started by addition of [^{14}C]-serotonin or [^{14}C]-phenylethylamine, as above. K_m (μM) and V_{max} (nanomoles per minute per mg of tissue) values were calculated by linear regression analyses of the Lineweaver-Burk plots.

In experiments on the interactive effects of several MAO inhibitors, these were mixed prior to incubation with the liver enzyme. Final concentrations of inhibitors examined in combination were the same as those used when the drugs were tested alone. Inhibition of MAO activity was expressed as decimal activity, which equals v_i/v_o, where v_i was activity in the presence of inhibitor(s) and v_o activity without inhibitor(s). To determine whether the inhibition produced by 2 or more drugs was additive, the decimal activity of the combination was compared with the product of the decimal activities obtained when each drug was assayed alone.

To examine the time-dependency of the inhibition of MAO by phentermine, 25 μl aliquots of rat brain, liver, and lung homogenates were preincubated at 37°C for 0, 5, 10, 15, 20, 25, 30 or 40 min with 100 μM of phentermine; [^{14}C]-5-HT (final concentration 125 μM) was then added and the mixtures were incubated for another 5 min. Reactions were stopped by adding 25 ml of 3 N HCl. Deaminated products were extracted and counted as above.

The reversibility of the inhibition of MAO by phentermine was studied using the dilution method. Rat liver was homogenized in 5 volumes of 0.1 M sodium-phosphate buffer (pH = 7.4) and aliquots (200 μl) of the homogenate were incubated at 37°C for 20 min in the absence or presence of phentermine (320 μM). After this preincubation, 50 μl of the mixture were diluted 50-fold with 0.1 M sodium phosphate buffer, pH 7.4, and the MAO activities in aliquots (50 μl) of the diluted homogenates, taken 5, 10, 15 or 20 min after dilution, were measured by incubating them with [^{14}C]-serotonin or [^{14}C]-phenylethylamine, as above. The degree of inhibition prior to dilution was determined by comparisons with MAO activities in aliquots of the undiluted homogenates.

In competition experiments, aliquots (25 μl) of rat brain homogenates were preincubated for 20 min at 37°C in the absence or presence of three concentrations of phentermine (100 μM, 320 μM and 1000 μM), in a volume of 50 μl. After preincubation, reactions were started by the addition of [^{14}C]-serotonin (final concentration, 31.3,62.5,125,250,500,1000 or 2000 μM), and incubation for 5 min. Reactions were stopped and the deaminated products extracted and counted as above. The enzyme activity (V; nmol/min/mg/tissue) observed using each concentration (S; μM) of substrate (serotonin) was calculated; double reciprocal plots were obtained by plotting 1/S values against 1/V values in the

absence or presence of phentermine.

Chemicals and drugs. [^{14}C]-serotonin creatine sulfate (1.8-2.2 Gbq/mmol) [^{14}C]-phenylethylamine hydrochloride (1.8-2.2 Gbq/mol.) were purchased from Amersham (Santa Clara, CA) and New England Nuclear (Boston, MA), respectively. Serotonin creatine sulfate, β-phenylethylamine hydrochloride, pargyline hydrochloride, clorgyline hydrochloride, iproniazid phosphate, phentermine HCl; *d*- and *l*-ephedrine; *d*- and *l*-pseudoephedrine; and *d,l*-norephedrine (phenylpropanolamine) and estradiol benzoate were purchased from Sigma Chemical Co. (St. Louis, MO). Nialamide, R(-)deprenyl hydrochloride, and tranylcypromine hydrochloride were purchased from Research Biochemicals International (Natick, MA).

RESULTS

Inhibition of MAO activities in rat lung, liver and brain by phentermine. Phentermine inhibited the ability of MAO to deaminate both serotonin and β-phenylethylamine in homogenates of rat lung, liver and brain. Inhibition occurred at micromolar concentrations, was dose-dependent, and was of roughly similar magnitude in all three tissues (Fig. 1). The drug was a more potent inhibitor of serotonin's metabolism than of phenylethylamine's (Table 1). Its inhibition of serotonin metabolism was enhanced by preincubation of the tissues (at

37°C), and was maximal and stable after 5 min of preincubation (Fig. 2). A similar time-dependence was also evident for the inhibition by phentermine of phenylethylamine metabolism.

Preincubation of rat liver homogenates with phentermine (320 μM) for 20 min inhibited the metabolism of both serotonin and phenylethylamine, by 87% and 48%, respectively. Diluting the samples 50-fold resulted in total recovery of MAO activities, indicating that phentermine inhibits MAO activities reversibly (Fig. 3). Moreover, as shown by kinetic studies this inhibition was competitive (Fig. 4).

Inhibition of MAO activities in rat lung, liver and brain by d-amphetamine and ephedrines. MAO activities in rat lung (Fig. 5), liver (Fig. 6), and brain (Fig. 7) were inhibited by *d*-amphetamine, *d*-ephedrine, *l*-ephedrine, *d,l*-norephedrine, *d*-pseudoephedrine, and *l*-pseudoephedrine at micromolar concentrations (Table 1), and in a concentration-dependent manner. In general, the responses of the three tissues were similar (Fig. 5-7), the inhibitory effects were reversible (Fig. 8), and the ability of each drug to inhibit the deamination of serotonin was greater than of phenylethylamine.

Inhibition of MAO activities in rat lung and brain homogenates by other drugs. To compare the inhibitory potencies of phentermine and other unlabeled MAO inhibitors with those of antidepressant MAO inhibitors, we characterized the

concentration-inhibition curves for the irreversible MAO inhibitors clorgyline, nialamide, deprenyl, tranylcypromine and phenelzine, as well as for iproniazid and moclobemide, in rat lung and brain homogenates. All of these compounds inhibited serotonin and phenylethylamine metabolism in both tissues, in a concentration-dependent manner (Figs. 9 + 10). Serotonin metabolism in lung (Fig. 9) and brain (Fig. 10) was inhibited by clorgyline, phenelzine, tranylcypromine, pargyline and deprenyl at nanomolar concentrations; as with phentermine, the inhibition produced by nialamide, moclobemide and iproniazid occurred only at micromolar concentrations. Phenylethylamine metabolism in lung (Fig. 9) and brain (Fig. 10) was inhibited by deprenyl, pargyline, tranylcypromine and phenelzine at nanomolar concentrations, and by clorgyline, nialamide, moclobemide and iproniazid at micromolar concentrations (Figs. 9 + 10).

The concentration-inhibition curves of iproniazid, nialamide and moclobemide in rat liver homogenates were similar to those of lung and brain homogenates (data not shown). The EC_{50} values for the MAO inhibitors, determined graphically from the concentration-inhibition curves, were similar (Table 2).

Interactions of phentermine and other MAO inhibitors.

Incubation with phentermine at a concentration, 130 μM, that alone caused 22% inhibition of hepatic MAO (serotonin-metabolizing) activity, caused considerably greater inhibition

when the incubation mixture also contained *l*-pseudoephedrine, *d*-pseudoephedrine, *l*-ephedrine, *d*-ephedrine, *d,l*-norephedrine, or estradiol benzoate. In all cases the magnitude of the inhibition observed with the drug combinations was close to, or equal to, the products of the inhibitions produced when the drugs were tested alone (Table 3). Similar additive effects were observed when phentermine was mixed with both *d*-pseudoephedrine and *d*-ephedrine, and when these three drugs were mixed with a fourth, *d,l*-norephedrine or estradiol benzoate. Additive effects were not observed when the above drugs were mixed with irreversible MAO inhibitors; in that situation the degree of inhibition obtained with the drug mixture was about the same as that caused by the irreversible MAO inhibitor alone. The fact that the effects of reversible MAO inhibitors are additive bears on the possible consequences, among obese European patients, of having taken both a fenfluramine and a multi-drug "compounded preparation" which frequently contained numerous MAO inhibitors, sometimes including *d*-amphetamine [18].

DISCUSSION

These data confirm earlier demonstrations [14,15] that phentermine is an MAO inhibitor, and show that it reversibly inhibits both the serotonin-metabolizing MAO-A and the β-phenylethylamine-metabolizing MAO-B (Fig. 1, Table 1). Its potency in inhibiting serotonin metabolism is on the same order as that of the antidepressant MAO inhibitors iproniazid and moclobemide (Table 2), but less than the potencies of such irreversible MAO inhibitors as pargyline and tranylcypromine (Fig. 9 + 10; Table 2). The effects on serotonin metabolism of combining phentermine with other widely-used MAO unlabeled inhibitors like pseudoephedrine, norephedrine, and estradiol are additive (Table 3); hence patients who took several of these compounds (and other MAOI, like *d*-amphetamine) in "compounded preparations" [18] may have experienced substantially greater MAO inhibition than that anticipated from the kinetic parameters of phentermine alone. The apparent potency of estradiol (i.e., estradiol benzoate) was low, perhaps reflecting the poor water-solubility of this hormone in the absence of estrogen-binding proteins. The hormone has been shown to exert anti-depressant effects, and these have been attributed to MAO inhibition [19].

The observed potency of phentermine for inhibiting the serotonin metabolizing MAO-A in rat liver, lung and brain, was much greater than that previously reported: The early studies concluded that phentermine inhibits MAO with EC_{50} values of 10 mm or 270 μM for rat liver [15] or brain [14], respectively. These

EC_{50} values were clearly different from, and much higher than, the K_i values (and apparent EC_{50} values) observed in the present study. In these previous studies, however, the concentrations of serotonin used as MAO substrate (5 mm or 2 mm) were several-fold higher than its concentration (125 μM) used in the present study. It is well known that MAO exists in two forms, MAO-A and MAO-B, and that the MAO-A preferentially metabolizes serotonin: the K_m values of serotonin for MAO-A and MAO-B are about 125 μM and 2 mm, respectively [20]. Thus, EC_{50} values of phentermine for the inhibition of MAO reported by Seiler and Wasserman [15] in liver, or by Nielsen and Dubnick [14] in brain are for MAO-A + MAO-B, but not MAO-A alone. Moreover, since the inhibition of MAO by phentermine is competitive (Fig.4), it is not possible to discern the effect of a competitive inhibitor (phentermine) when the substrate concentration (serotonin) is 8- to 20-fold higher than the K_m value of the competitor. Clearly the potency of phentermine was underestimated in the previous studies.

Phentermine was second only to *d*-amphetamine, among the unlabeled MAO inhibitors tested in the present study, for its ability to inhibit MAO-A as well as MAO-B. The potencies of phentermine, d-amphetamine and the related drugs for inhibiting the serotonin metabolizing MAO-A were higher than their potencies for inhibiting the β-phenylethylamine-metabolizing MAO-B in each of the rat tissues tested (Table 1). These results indicate that phentermine, *d*-amphetamine, and the other drugs tested are preferentially MAO-A inhibitors. In agreement with the present

results, it has been reported previously that d-amphetamine [21], various amphetamine analogs [22], and d,l-norephedrine [23] preferentially inhibit MAO-A. The reported K_i values for d-amphetamine, 20 μM [22] and 11 μM [22] and for d,l-norephedrine, 150 μM [23], are also in good accordance with those found in the present study.

What is the contribution of phentermine's MAO-A inhibiting activity to its overall pharmacological effects (i.e., its biochemical, behavioral, and toxic actions), when given alone or in combination with other drugs? In central and peripheral catecholaminergic synapses phentermine interacts with noradrenaline and dopamine transporters at about 2-8 μM [24,25] to increase the levels of these transmitters; it thereby produces "sympathomimetic", "central stimulating" and anorectic effects. The inhibition of MAO-A by phentermine would be expected to enhance these pharmacological effects by slowing the oxidative deamination of intraneuronal catecholamines and of extraneuronal catecholamines released from pre-synaptic sites. Thus, phentermine's inhibition of MAO-A might contribute to its pharmacological effects; however because the transmitters it influences through MAO-A inhibition are the same as those released by the drug, qualitative differences would not be expected. The severe side effects of classical irreversible MAO inhibitors, such as the hypertensive crisis observed after ingestion of tyramine -rich foods (e.g., cheeses), is also unlikely to occur with phentermine treatment because the MAO

inhibition it produces is reversible and is relatively selective.

When phentermine-induced MAO-A inhibition occurs concurrent with serotonin uptake blockade (caused by fluoxetine) and/or serotonin release (by fenfluramine or dexfenfluramine), the observed effects might differ qualitatively from those of either drug given alone. Package inserts and product labels for fenfluramine and dexfenfluramine- as well as for all of the other serotonin-reuptake blockers [9]- have stated unequivocally that these drugs are never to be administered along with an MAOI, lest free serotonin in brain or blood rise to toxic levels and produce a "serotonin syndrome" or pathologic changes in vascular tissue. In two important sites - the central nervous system and the lung - the combination of phentermine and a serotonin uptake blocker or releaser might be expected to cause major, if transient, increases in free (i.e., plasma or intrasynaptic) serotonin. Neurochemical experiments show that MAO inhibition per se can increase extracellular serotonin [26] and enhance such serotonin mediated effects as anorexia [27]. Similarly, phentermine alone suppresses appetite [28] and increases extracellular serotonin levels [29,30] and when given in combination with serotoninergic agents like fenfluramine, enhances their effects on appetite [31] and on extracellular serotonin levels [30]. Phentermine's effects on extracellular brain serotonin levels, when given alone or combined with fenfluramine [29,30] are compatible with its inhibitory action on MAO-A (Table 1). The reversible and competitive nature of the MAO-A inhibition produced by

phentermine may help to explain why the phentermine-fenfluramine combination, while more effective than fenfluramine alone in treating obesity, was associated with relatively few cases of "serotonin syndrome", or with the vascular damage seen when strong and irreversible MAOIs are combined with serotonin uptake blockers or releasers. Perhaps, because phentermine failed to produce severe, acute toxic reactions when taken with tyramine-rich foods or serotonin uptake blockers, its ability to inhibit MAO was thought to be relatively unimportant in its pharmacological actions, and so this inhibition went generally unrecognized after its initial description. Even though this action was initially described and confirmed in the 1970's [14,15], the drug's package insert never mentioned it, and few if any physicians were (or are) aware of it. This situation allowed millions of Americans to take phentermine in combination with fenfluramine (as "fen-phen") [8], and also allows some patients to take "pro-phen" (Prozac, or fluoxetine, plus phentermine) right now.

The effect of phentermine as a MAO-A inhibitor on peripheral serotonin metabolism, mainly in the lung, may explain the cardiac valvular lesions [1,2] and perhaps the primary pulmonary hypertension [18] observed in some patients taking "fen-phen". The lung is uniquely important in peripheral serotonin metabolism in both rats [16] and humans [32]. Most if not all, circulating serotonin is cleared by the lung, through active uptake into lung tissue followed by oxidative deamination [16,32]. An important

functional consequence of this inactivation is that reduced concentrations of serotonin thus enter the left atrium and systemic circulation. The inhibition of MAO-A in lung tissue by phentermine and related drugs strongly suggests that these drugs thereby inhibit serotonin metabolism, and allow more intact serotonin to enter the left atrium. This would be especially likely when phentermine or other unrecognized MAOIs were given in combination with a serotonin uptake blocker (which would inhibit the uptake of serotonin by lung tissue) or a serotonin releaser. The only way to prove conclusively that giving a serotonin uptake inhibitor with phentermine increases pulmonary plasma serotonin concentrations would be to give the drugs to volunteers and measure serotonin and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in plasma samples obtained from pulmonary arterial and venous blood. But at the present such a study would be unethical. Data are available, however, on the effect of giving phentermine to rats, without serotonin uptake blockers, on serotonin metabolism in lung [33]. A phentermine dose of 0.5 mg/kg decreased the conversion of serotonin to 5-HIAA by more than half, and a 10 mg/kg dose blocked this conversion almost completely. Administration of the 0.5 mg/kg phentermine dose generates lung phentermine concentration of about 60 μM (i.e., 19 μg/lung of drug, and 3 g of lung tissue), while the 10 mg/kg dose generates concentrations as high as 500 μM. The ability of a phentermine dose to suppress the clearance of pulmonary serotonin was found to be proportional to the tissue phentermine

concentration [33]. It would be anticipated that giving phentermine with a serotonin uptake blocker, like fenfluramine, would amplify this effect, inasmuch as the serotonin in pulmonary plasma would no longer be protected from oxidative deamination by being taken up by platelets. Such an increase in the concentration of blood serotonin leaving the lung would be expected to increase pulmonary vascular resistance, as has been demonstrated in the isolated perfused rat lung [34]. In accordance with this view, at least one case of primary pulmonary hypertension has already been described in a patient chronically taking phentermine along with the serotonin-uptake blocker fluoxetine [35].

When investigators at the Mayo Clinic first described surgically-confirmed heart valve lesions in people who had been treated with "fen-phen" [1], and when the FDA, analyzing uncontrolled echocardiographic data from five laboratories, concluded that as many as 30% of patients so treated went on to develop such lesions [2], it was speculated that the lesions resulted from pathologic elevations in blood serotonin, similar to those seen in carcinoid syndrome, which had been caused by the fenfluramines [1]. (Fenfluramines do block serotonin's reuptake, and their metabolites the norfenfluramines do release the indoleamine; however their administration without an MAOI does not elevate serotonin levels in blood cells or in plasma [36].) A single group of investigators also presented echocardiographic evidence [2,37] that heart valve lesions had developed in a few

patients who had taken dexfenfluramine without phentermine, and on that basis the companies marketing dexfenfluramine or fenfluramine voluntarily withdrew them. Phentermine - which, by itself, had not been associated with valvular lesions - continued to be available. That fenfluramine or dexfenfluramine, administered alone, could generate such lesions might have been questioned given their apparent absence in the tens of millions of patients outside the United States who had been treated, since the 1960's, with one or the other drug; it might instead have been hypothesized that this new, uniquely-American clinical problem was related to the uniquely-American way that fenfluramine usually was administered, i.e., in combination with phentermine.

Phentermine was and continues to be described in its package insert as a sympathomimetic amine [9]. Very few articles have been published on the drug's pharmacologic effects during the past two decades, and - as far as we can determine - no articles have confirmed its safety, in humans or experimental animals, when taken in combination with a fenfluramine. We observe that a number of widely-used over-the-counter or prescription drugs besides phentermine also inhibit MAO-A, e.g., pseudoephedrine, norephedrine, amphetamine, ephedrine, and estrogen (Tables 2 + 3). Most of these compounds are also reversible MAOI, and when two or more are combined their effects on MAO are additive (Table 4). Since some patients probably took these drugs on a daily basis for extended periods - perhaps without informing their

physicians - their use might explain the very small number of patients who reportedly developed valvular lesions after taking "only" a fenfluramine. (The converse situation probably was less likely to occur: While many drugs turn out to be unrecognized MAOI's, few if any are unrecognized serotonin-uptake blockers; hence it is highly unlikely that a patient taking phentermine or another unlabeled MAOI would also unknowingly have taken a serotonin-uptake blocker.)

The effects of unrecognized MAOI's on pulmonary serotonin metabolism might also explain the tendency of fenfluramine-associated valvular lesions to affect the left side of the heart[1] - in contrast to the right-sided lesions found in patients with the carcinoid syndrome - as well as the suggested association between fenfluramine use and primary pulmonary hypertension [18]: Most circulating serotonin is cleared in the lung, through metabolism by MAO [16]. In the carcinoid patient pulmonary MAO is still active, so plasma serotonin concentrations in the right side of the heart are greater than those on the left side; however, as discussed above, in patients taking an MAOI the pulmonary clearance of serotonin is inhibited, so the difference between right- and left-sided plasma serotonin levels may disappear, and valvular lesions become as likely to develop in the left side. In the case-control study [18] that associated fenfluramine use with primary pulmonary hypertension, the initial analysis concluded that 20 of the 95 patients had taken an anorexic drug, e.g., fenfluramine or dexfenfluramine, yielding a

barely-significant odds ratio. However the investigators later transferred 10 additional patients from the 95 into the "anorexic drug" category, elevating the odds ratio (for patients who took a fenfluramine during the preceding year) to 10.1. Seven of these ten were known also to have taken the "compounded preparations", discussed above, which included a number of drugs now demonstrated as having MAOI activity, and the other three lacked adequate pharmacy records; thus the factor that increased the incidence might have been the concurrent use, chronically, of a fenfluramine with one or more MAOI. In a recent multicenter study on the association of dexfenfluramine use and cardiac valvular lesions, significant differences between drug-treated and matched control groups disappeared when the patients who had also taken an MAO-inhibiting agent were omitted from the analysis [39]. Reanalysis of the case-control data on primary pulmonary hypertension [18] to include only patients not also taking an MAOI might similarly remove differences between fenfluramine-treated and control groups.

Clearly, something must be done to protect patients from using novel drug combinations - like "fen/phen", or now phentermine plus fluoxetine (Prozac) ("phen-pro") - the safety of which has not been affirmed, and whose components may not even have been accurately labeled. In the interim, steps must be taken to affirm that <u>all</u> drugs that inhibit serotonin metabolism by MAO are labeled as such, and that physicians be warned once again not to combine such drugs with serotonin-uptake blockers.

ACKNOWLEDGMENTS:

These studies were supported in part by grants from the Center for Brain Sciences & Metabolism Charitable Trust. We thank J.P. Shi for valuable technical assistance.

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Table 1: K_i values of phentermine and some related drugs for MAO-A and MAO-B in rat lung, liver and brain homogenates.

	K_i (μM)					
	MAO-A			MAO-B		
Drugs	Lung	Liver	Brain	Lung	Liver	Brain
Phentermine	76 ± 4	80 ± 7	89 ± 6	326 ± 43	416 ± 57	310 ± 90
d-Amphetamine	6.1 ± 0.6	6.5 ± 0.8	4.9 ± 0.3	106 ± 9	119 ± 8	118 ± 21
d,l-Norephedrine	243 ± 33	267 ± 16	222 ± 18	3400 ± 650	3300 ± 850	2600 ± 820
d-Ephedrine	840 ± 60	844 ± 41	644 ± 43	5500 ± 200	5500 ± 650	5500 ± 120
l-Ephedrine	570 ± 50	600 ± 54	520 ± 19	4250 ± 750	4700 ± 560	4600 ± 400
d-Pseudoephedrine	1220 ± 120	1030 ± 110	1020 ± 30	5750 ± 750	5200 ± 600	5200 ± 400
l-Pseudoephedrine	890 ± 20	870 ± 50	970 ± 20	<6500	<6500	<6500

MAO-A and MAO-B activities were assayed using serotonin and phenethylamine, respectively, as substrates, and concentration-inhibition curves were obtained for the drugs indicated in rat lung, liver, brain homogenates as described in Figure 1. The concentration producing 50% inhibition (IC_{50}) was calculated graphically from semi-log plots (the concentration-inhibition curve) of inhibitor concentration against percentage inhibition. K_i values (μM) were determined from the equation $K_i = IC_{50}/1 + S/Km$). S value (substrate concentration as μM) in this equation was 125 μM for MAO-A or 8 μM for MAO-B respectively. K_m values (μM) of serotonin (MAO-A) or ß-phenethylamine; (MAO-B) were obtained from double-reciprocal Lineweaver-Burk plots in kinetic experiments for each tissue. Data are given as mean ± S.E. of 4-7 determinations.

Table 2: EC_{50} values of MAO inhibitors for MAO-A and MAO-B in rat lung and brain homogenates.

	$EC_{50}(\mu M)$			
	MAO-A		MAO-B	
Inhibitors	Lung	Brain	Lung	Brain
Clorgyline	0.005 ± 0.001	0.006 ± 0.001	11 ± 3	6 ± 2
Nialamide	3.0 ± 0.2	2.6 ± 0.4	21 ± 5	13 ± 1
Deprenyl	1.5 ± 0.2	1.3 ± 0.2	0.011 ± 0.002	0.021 ± 0.006
Tranylcpromine	0.222 ± 0.29	0.227 ± 0.046	0.156 ± 0.022	0.087 ± 0.016
Phenelzine	0.322 ± 0.050	0.310 ± 0.050	0.380 ± 0.040	0.197 ± 0.016
Parygyline	0.810 ± 0.140	0.700 ± 0.110	0.027 ± 0.003	0.019 ± 0.002
Moclobemide	22 ± 3	32 ± 5	<3200	<3200
Iproniazide	78 ± 18	59 ± 14	59 ± 14	48 ± 10

MAO-A and MAO-B activities were assayed and the concentration-inhibition curves obtained for their inhibition by known MAO-A and MAO-B inhibitors in lung and brain homogenates. The concentration producing 50% inhibition (IC_{50}) was calculated graphically from semi-log plots (the concentration-inhibition curve) of inhibitor concentration against percentage inhibition. Data are given as means ± S.E. of four determinations.

Table 3: Inhibition of Rat Liver MAO-A by Combinations of Inhibitors

Treatment	Inhibitor	conc. (mM)	MAO ACTIVITY	
			Observed	Predicted
1	phentermine	0.13	0.78	
2	*l*-pseudoephedrine	0.89	0.74	
3	*d*-pseudoephedrine	1.33	0.79	
4	*l*-ephedrine	0.89	0.74	
5	*d*-ephedrine	1.00	0.83	
6	*d,l*-norephedrine	0.33	0.77	
1+2			0.57	0.58
1+3			0.63	0.62
1+4			0.58	0.58
1+5			0.67	0.65
1+6			0.61	0.60
1	phentermine	0.12	0.79	
7	estradiol benzoate	0.20	0.69	
1+7			0.54	0.54
1	phentermine	0.13	0.78	
3	*d*-pseudoephedrine	1.33	0.79	
5	*d*-ephedrine	1.67	0.73	
1+3+5			0.47	0.45
1	phentermine	0.075	0.89	
3	*d*-pseudoephedrine	1.00	0.81	
5	*d*-ephedrine	1.00	0.83	
6	*d,l*-norephedrine	0.25	0.77	
1+3+5+6			0.47	0.46

Rat liver homogenates were incubated with serotonin plus one or more drugs, and MAO-A was assayed as described in the text. In studies on estradiol benzoate, the media contained DMSO to increase this agent's solubility. Predicted MAO activity was obtained from the products of the observed activities for each of the drugs when present alone.

Figure 1. Concentration-inhibition curves for inhibition by phentermine of MAO activities in homogenates of rat lung, liver and brain. Aliquots (25 μl) of the homogenates were preincubated for 20 min at 37°C in the absence or presence of various concentrations of phentermine (10,32, 100,320,1000, 3200, 10000 and 32000 μM) in a volume of 50 μl. After the preincubation period, the reaction was started by the addition of [^{14}C]-serotonin (final concentration, 125 μM) or [^{14}C]-phenylethylamine (final concentration, 8 μM), and the assays continued as described in the text. Enzyme activities in the presence of each drug concentration were expressed as percents of those in homogenates preincubated without phentermine (control). Concentration-inhibition curves were obtained by plotting log molar concentrations of phentermine against percent enzyme activity. Each point represents the mean ± S.E. of 5-7 experiments.

Figure 2. Time course of the inhibition by phentermine of serotonin metabolism in rat lung (top), liver (middle) and brain (bottom) homogenates. Assays were performed and analyzed as described in the text. Each point represents the mean ± S.E. of 3 experiments.

Figure 3. Reversibility by dilution of the inhibition by phentermine of serotonin and phenylethylamine metabolism in rat liver. Rat liver was homogenized in 5 volumes of 0.1 M sodium-phosphate buffer (pH = 7.4) and aliquots (200 μl) of the homogenate were incubated at 37°C for 20 min in the absence or presence of phentermine. After the preincubation, 50 μl of the mixture were diluted 50-fold with 0.1 M sodium phosphate buffer, pH 7.4, and aliquot (50 μl) of the diluted homogenates were taken 5,10,15 or 20 min after dilution, and incubated with [^{14}C]-serotonin (125 µM) or [^{14}C]-phenylethylamine (8 μM) as described in the text. Activities were compared with those of undiluted homogenates. Each point represents the mean ± S.E. of 3 experiments.

Figure 4. Double reciprocal plot of the inhibition by phentermine of [^{14}C]-serotonin deamination in rat brain homogenates. Aliquots (25 μl of rat brain homogenates were preincubated for 20 min at 37°C in the absence (●) or in the presence of 100 μM(o), 320 μM (v) or 1000 μM (v) of phentermine in a volume of 50 μl. After the preincubation, the reaction was started by the addition of [^{14}C]-serotonin (final concentration, 31.3, 62.5,125,250,500,1000 or 2000 μM), and incubated for 5 min. The rest of the assay was performed as described in the text. Double reciprocal plots were obtained by plotting the 1/S value against the I/V value in the absence or presence of phentermine. Each point represents the mean of duplicate determinations of one typical experiment.

Figure 5. Concentration-inhibition curves in rat lung homogenates of some sympathomimetic drugs. Each point represents the mean ± S.E. of 4 experiments.

Figure 6. Concentration-inhibition curves in rat liver homogenates of some sympathomimetic drugs. Each point represents the mean ± S.E. of 4 experiments.

Figure 7. Concentration-inhibition curves in rat brain homogenates of some sympathomimetic drugs. Each point represents the mean ± S.E. of 4 experiments.

Figure 8. Reversibility of inhibition by various sympathomimetic drugs of serotonin-metabolizing activity in rat liver homogenates. Each point represents the mean of duplicate determinations of one typical experiment.

Figure 9. Concentration-inhibition curve in rat lung homogenates of some MAO inhibitors. Each point represents the mean ± S.E. of 4 experiments.

Figure 10. Concentration-inhibition curves in rat brain homogenates of some MAO inhibitors. Each point represents the mean ± S.E. of 4 experiments.

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